Regulation of Eukaryotic Abasic Endonucleases and Their Role in Genetic Stability

Bruce Demple, Lynn Harrison, David M. Wilson III, Richard A.O. Bennett, Toshimitsu Takagi, and A. Gian Ascione

Department of Molecular and Cellular Toxicology, Harvard School of Public Health, Boston, Massachusetts

Abasic (AP) sites in DNA arise from spontaneous reactions or the action of DNA glycosylases and represent a loss of genetic information. The AP sites can be mutagenic or cytotoxic, and their repair is initiated by class II AP endonucleases, which incise immediately 5′ to AP sites. The main enzyme of *S. cerevisiae*, Apn1, provides cellular resistance to oxidants (e.g., H_2O_2) or alkylating agents, and limits the spontaneous mutation rate. AP endonucleases from other species can replace Apn1 function in yeast to different extents. We studied the main human enzyme, Ape, with respect to its incision specificity *in vitro* and the expression of the *APE* gene *in vivo*. The results suggest that Ape evolved to act preferentially on AP sites compared to deoxyribose fragments located at oxidative strand breaks and that the incision modes of Ape and Apn1 may be fundamentally different. We also defined the functional *APE* promoter, and showed that *APE* expression is transiently downregulated during the regeneration of epidermis after wounding. This latter effect may lead to a window of vulnerability for DNA damage and perhaps mutagenesis during the healing of epidermal and other wounds. Such unexpected effects on the expression of DNA repair enzymes need to be taken into account in analyzing the susceptibility of different tissues to carcinogens. — *Environ Health Perspect* 105(Suppl 4):931–934 (1997)

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Introduction

Sites of base loss in DNA (abasic [AP] sites) occur frequently in living cells. For example, AP sites arise spontaneously through acid-catalyzed purine hydrolysis at an estimated rate of approximately 10^4 per human genome per day (*1*). To this burden is added the removal of various types of damaged bases via DNA glycosylases and the direct elimination of bases via free-radical attack (*1*,*2*). The resulting AP sites have both cytotoxic and mutagenic potential, which is countered by the action of AP endonucleases that initiate their repair. The most abundant activities in most cells are class II AP endonucleases, which initiate repair by incising DNA immediately 5′ to AP sites (*2*). Some DNA glycosylases associate with AP lyase activity that results in the incision of DNA immediately 3′ to the AP site via β-elimination. These class I enzymes are always associated with DNA glycosylases, but the biological function of AP lyase activity remains to be established (*2*).

Enzymatic and molecular genetic studies have revealed that class II AP endonucleases are comprised of two phylogenetic groups, each of which includes both prokaryotic and eukaryotic members (*2*). One family includes *Escherichia coli* endonuclease IV and *Saccharomyces cerevisiae* Apn1 protein, in addition to predicted proteins from *Schizosaccharomyces pombe*, *Caenorhabditis elegans*, and *Mycoplasma genitalium* (Table 1). Members of the other class II AP endonuclease family are related to *E. coli* exonuclease III, with homologous proteins or segments found for Gram-positive bacteria, *Drosophila melanogaster*, *Arabidopsis thaliana*, *Dictyostelium discoideum*, *S. cerevisiae*, and mammalian cells (Table 1). Many AP endonucleases also display 3′-repair diesterase activities that liberate nucleotide fragments from DNA 3′ termini (*2*). Studies of enzymes from both families have demonstrated their roles in repairing DNA damage due to environmental agents and in limiting spontaneous mutation in the face of DNA damages arising from endogenous sources.

Apn1 and Genetic Stability in Yeast

Apn1 protein of *S. cerevisiae* was first isolated as the major 3′-repair activity in yeast, but was shown also to be the predominant AP endonuclease of that organism. The availability of the purified protein soon led to cloning and sequencing of the structural gene *APN1*, which revealed the homology of the yeast protein to *E. coli* endonuclease IV (*3*). Yeast *apn1*$^-$ strains were constructed and found to be hypersensitive to the cytotoxicity of various agents. These observations revealed roles for Apn1

Table 1. Homologous class II abasic endonuclease families.

Organism (reference)	Endonuclease IV family homolog	Exonuclease III family homolog
S. pneumoniae (*2*)	—	Exonuclease A
*C. burnetii orf*208 (*16*)	—	Exonuclease III-like
H. influenzae (*7*)	—	Exonuclease III-like
M. genitalium (*8*)	Endonuclease IV-like	—
S. cerevisiae YBL019W (*19*)	Apn1	Exonuclease III-like
S. pombe (*20*)	Apn1	—
C. elegans (*21*)	Apn1-like	—
A. thaliana (*22*)	—	Arp
D. melanogaster (*2*)	—	Rrp1
D. discoideum (*23*)	—	ApeA
R. norvegicus (*24*)	—	Apen
M. musculus (*2*)	—	Apex
B. taurus (*2*)	—	Bap1
H. sapiens (*2*)	—	Ape/Hap1/Apex/Ref-1

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Address correspondence to Dr. B. Demple, Department of Molecular and Cellular Toxicology, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115. Telephone: (617) 432-3462. Fax: (617) 432-0377. E-mail: demple@mbcrr.harvard.edu

Abbreviation used: AP, abasic or apurinic/apyrimidinic.

protein in repairing DNA damages caused both by simple alkylating agents (e.g., methyl methanesulfonate), probably AP sites, and by chemical oxidants (e.g., H_2O_2), probably oxidized nucleotide fragments blocking 3′ termini at oxidative strand breaks (*4*).

Apn1-deficient yeast also exhibit an elevated rate of spontaneous mutation (*4*). This *apn1*$^-$ mutator effect is seen under both aerobic and anaerobic conditions, which attests to the mutagenic potential of both oxidative and nonoxidative metabolic by-products of normal metabolism. Aerobically, the *apn1*$^-$ mutator drives AT to CG transversions at an approximately 60-fold higher rate than found for wild-type cells (*5*). Thus, a high rate of this otherwise rare mutation might be a general indicator of deficiency in the repair of AP sites. Apn1-deficient yeast also provides a eukaryotic vehicle for analyzing the *in vivo* functions of enzymes from other organisms.

Enzymology of Human Abasic Endonuclease

Our studies initially focused on mammalian enzymes that remove 3′-damages from a synthetic substrate; this approach identified in various mammalian cell types (e.g., HeLa, CHO, and human fibroblasts) and in calf thymus two main 3′-repair activities separable by chromatography on BioRex-70 (Bio-Rad, Richmond, CA) (*6*). The responsible proteins were both purified extensively from HeLa cells, one (BioRex Peak II) to apparent physical homogeneity (*6*). Robust class II AP endonuclease was present in both preparations, at approximately 7-fold higher than the 3′-repair activity for Peak I and approximately 200-fold for Peak II. The purity of the Peak II enzyme (M_r 37,000) allowed the determination of 25 residues of its *N*-terminal sequence and the generation of high-affinity polyclonal antibodies, reagents that were used to clone and verify the cDNA encoding this protein, which was named APE (*7*). The translated APE cDNA sequence revealed a homolog of *E. coli* exonuclease III, with 28% sequence identity between the two proteins over a 280-residue segment (i.e., virtually the entire length of exonuclease III). This homology was confirmed by independent cloning efforts (*8,9*). Together with proteins from Gram-positive bacteria, plants, *Drosophila*, and other mammalian cells, Ape protein (also called Hap1, Apex, or Ref-1) forms a highly conserved family of AP endonucleases (Table 1).

The enzymatic specificity of purified Ape protein has been examined in some detail. The human protein has an approximately 10-fold higher cleavage rate for AP sites than does exonuclease III, but with far weaker 3′-repair diesterase and 3′-phosphatase activities (*6*). This *in vitro* pattern is consistent with the behavior of Ape in transcomplementation studies (below). Ape's enzymatic specificity has more recently been examined for a series of synthetic abasic site analogs (Figure 1) and in a variety of structural contexts (*10*). These studies show several important features of the recognition and cleavage mechanism of this enzyme: *a*) Ape has no obvious requirement for the deoxyribose ring structure (efficient cleavage of the propanediol derivative) (Table 2); *b*) Ape is relatively insensitive to shortening of the

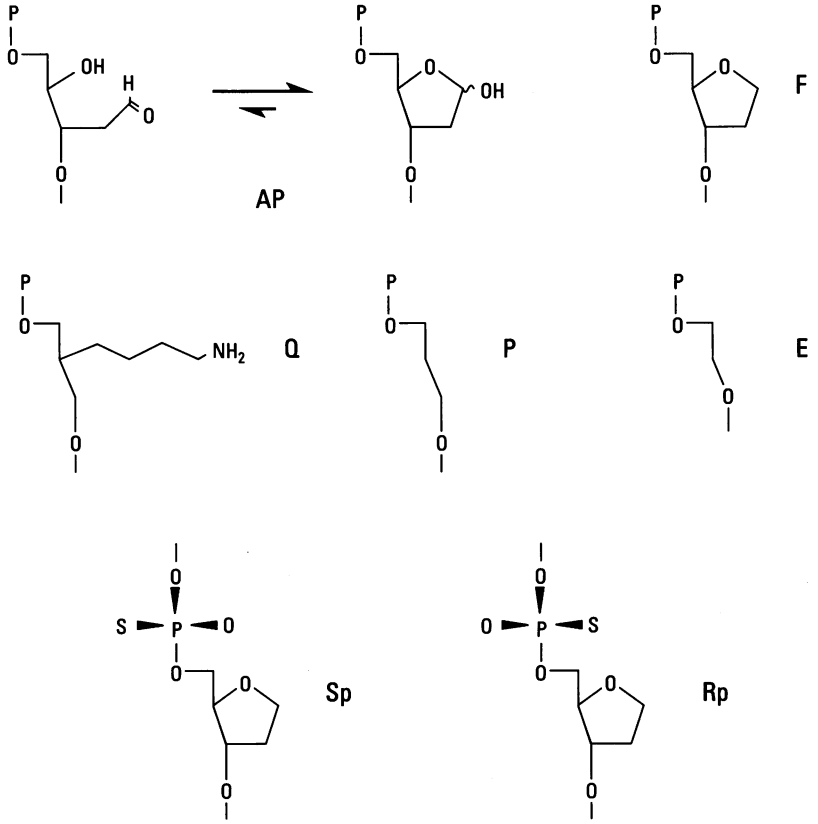

Figure 1. Structures of synthetic abasic sites. These sites were positioned in duplex synthetic oligonucleotides of 18 to 23 bp, with the substrates labeled at the 5′ end of the strand bearing the abasic site. Abbreviations: AP, a hydrolytic apurinic/apyrimidinic site, as would be produced by spontaneous hydrolysis or the action of DNA glycosylases; F, tetrahydrofuranyl residue; Q, 2-(aminobutyl)-1,3-propanediol residue; P, 1,3-propanediol residue; E, ethanediol residue; Sp and Rp, phosphorothioate diastereomers positioned 5′ to F residues. Reprinted from Wilson et al. (*10*), with permission.

Table 2. Specificities of class II abasic endonucleases.

Abasic site analog[a]	Relative incision rate: *E. coli* endonuclease IV	*S. cerevisiae* Apn1 protein	*E. coli* exonuclease III	*H. sapiens* Ape protein
F	≡1	≡1	≡1	≡1
P	3.3	2.4	0.8	2
E	0.1	0.1	0.2	1
Rp	<0.001	<0.003	<0.01	0.05
Sp	0.4	1.2	<0.01	<0.0001
Q	0.1	0.8	0.001	0.003

Abbreviations: F, tetrahydrofuranyl residue; P, 1,3-propanediol residue; E, ethanediol residue; Rp and Sp, phosphorothioate diastereomers positioned 5′ to F residues; Q, 2-(aminobutyl)-1,3-propanediol residue. [a]Data from Takeuchi et al. (*25*), Wilson et al. (*10*), and Wilson et al. (unpublished data).

phosphodiester spacing across the abasic site (efficient cleavage of the ethanediol derivative); *c*) Ape does not cleave when the abasic 4′-carbon is branched (Q substrate); *d*) Ape has a stronger requirement for properly base-paired duplex DNA on the 5′ than on the 3′ side of an abasic site; *e*) Ape acts poorly on abasic sites located close to blunt termini (poor cleavage if there are <5 base pairs on the 5′ side or <4 base pairs on the 3′ side; *f*) Ape displays stereospecificity for inhibition of cleavage by phosphorothioate residues at the 5′ side of an abasic site (Rp is a significant substrate, Sp is not). Ape protein harbors a weak intrinsic 3′-exonuclease function, which is present at approximately 10^{-4} of its AP endonuclease activity and strongly dependent on the substrate and the reaction conditions (*10*). The results are consistent with a model in which Ape recognizes substrates by the absence of a base in the context of normal duplex DNA 5′ to the site, followed by stereospecific cleavage of the 5′-phosphodiester bond. This pattern of specificity is for the most part reflected in the activities of *E. coli* exonuclease III, but is significantly different than the substrate specificity of the nonhomologous proteins endonuclease IV of *E. coli* and yeast Apn1 (Table 2). These *in vitro* differences and the structural unrelatedness of these proteins echo their specificities for DNA repair *in vivo*.

Some of our recent studies have been directed at dissecting the mechanistic steps of substrate recognition and cleavage by Ape. DNA binding studies have revealed a strong specificity of Ape for AP sites under conditions in which cleavage is inhibited (in the presence of metal chelators) and have allowed us to observe protein–DNA complexes by both gel electrophoresis and filter-binding methods. Such complexes can account for up to one-third of the total DNA present (with the F substrate, for example), and appear to reflect the normal cleavage pathway because the DNA is rapidly cleaved upon the addition of Mg^{2+} to support the reaction. The details of Ape's interaction with DNA have been explored using a variety of footprinting methods, which reveal the way this enzyme binds its substrates for cleavage (*11*).

The *in vitro* specificity of Ape is reflected in the ability of the protein to effect repair in transcomplementation studies. Our original studies showed that expression of Ape in AP endonuclease-deficient *E. coli* conferred resistance to alkylating agents but not to the oxidant hydrogen peroxide (*7*). A similar specificity has now been demonstrated for Apn1-deficient yeast (*12*). These *in vivo* effects are consistent with the robust activity of Ape as an AP endonuclease coupled to a weak 3′-repair diesterase, which would effect the efficient repair of alkylation-induced AP sites but not of oxidative strand breaks in DNA (*2*). Most importantly, expression of Ape in Δ*apn1* yeast at a modest level (~2000 Ape molecules per cell, compared to ~7000 Apn1 molecules per cell in wild-type yeast) was sufficient to restore a normal spontaneous mutation rate (*12*). This observation underscores the potential of endogenously generated AP sites to cause genetic instability when they are left unrepaired.

Biology of *APE* in Mammalian Cells

Although the role of Ape protein in DNA repair is likely to be a broad one, neither the active enzyme nor its mRNA were inducible by a variety of DNA-damaging agents (*13*). We addressed the possibility that the *APE* gene might be regulated during the cell cycle or in response to proliferation, under the assumption that Ape protein would help eliminate mutagenic damage from DNA to allow accurate replication. For this purpose, we examined the expression of *APE* mRNA by *in situ* hybridization in porcine skin following surgical wounding (*14*). In this model, robust proliferation of keratinocytes begins approximately 12 hr after wounding and continues for 3 to 4 days until the wound site is filled; differentiation over the next 10 to 15 days then generates the layers of normal skin. During this period the expression of genes encoding certain growth factors and their receptors is induced, while others remain unaffected.

For expression of *APE* mRNA, we found no significant change during the first 6 hr after wounding, followed by a 3-fold decrease at day 1 that persisted into day 2; by day 3, expression began to recover, was near normal on day 5, exceeded the level seen for unwounded skin at day 9, and was still more strongly elevated at day 17 (Figure 2). Given the foregoing rationale, this biphasic pattern of expression that was negatively correlated with cell proliferation was quite unexpected. These observations may suggest that Ape activity is not limiting for essential DNA repair under the cell growth conditions of wound healing in the porcine skin model, and have prompted a consideration of alternative explanations for the observed biphasic regulation (*14*). One possibility is that Ape in some way interferes with rapid proliferation and must be removed; such a possibility seems inconsistent with the large amount of Ape protein present in rapidly proliferating HeLa cells. Alternatively, *APE* expression might be regulated for functions unrelated to DNA repair, such as the redox-regulatory role proposed by Xanthoudakis et al. (*15*). Still another possibility is that *APE* is regulated for its repair functions, but with a greater role in differentiated skin than during wound healing. For example, Ape could assist in the repair of oxidative damage caused by solar radiation. In the latter scenario, healing wounds might be especially vulnerable to certain kinds of DNA damage during the period when *APE* expression is diminished. Future experiments must focus on the expression of the *APE* gene during the differentiation of keratinocytes *in vitro* and establish whether *APE* regulation occurs transcriptionally or posttranscriptionally.

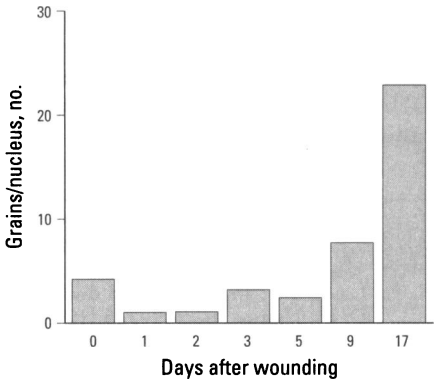

Figure 2. Biphasic *APE* expression during wound healing. Porcine *APE* mRNA was detected by *in situ* hybridization with an antisense-strand riboprobe generated from APE cDNA. Expression was quantitated by scoring the mean number of autoradiographic grains per cell for representative fields from the indicated times after wounding. Reprinted from Harrison et al. (*14*), with permission.

Conclusion

AP endonucleases constitute a major cellular defense against genetic damage caused by a wide variety of agents both environmental and intrinsic to normal metabolism. The main enzyme of human cells, encoded by the *APE* gene, belongs to a protein family that includes exonuclease III of *E. coli*; these enzymes share substrate specificity that distinguishes them from the unrelated AP endonucleases encoded by the *E. coli nfo* and yeast *APN1* genes (Table 2). The main activities of Ape protein are directed at AP sites in DNA, with

some indication that this specificity is determined at the level of DNA binding. Although the *APE* gene is not induced by DNA-damaging agents that generate free radicals or alkylated bases, the *APE* transcript is regulated during tissue regeneration that follows surgical wounding in skin. The biphasic expression seen during wound healing could render healing skin transiently susceptible to genetic damage, or it could be part of an overall program of differentiation in epidermis. Ape protein is clearly a vital cellular component whose biological roles are just beginning to be understood.

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